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EXAMINER	
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ART UNIT	**PAPER NUMBER**
1637	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/749,527	KOO ET AL.	
	Examiner	Art Unit	
	Angela Bertagna	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *26 July 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-48* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-48* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

FINAL REJECTION

Remarks

1. Claims 1-48 are currently pending. In the response filed July 26, 2006, claims 1, 16-19, 23-27, 29-30, 33-36, 39, and 41 were amended. Claims 45-48 are new.

New Grounds of Rejection Necessitated by Applicant's Amendment

Claim Rejections - 35 USC § 112 - Enablement

2. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-15, 18-22, and 45 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter that was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CA FC 1988). Wands states at page 1404,

> "Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

Nature of the Invention & Breadth of the Claims

The invention relates to methods of sequencing nucleic acids based on surface-enhanced Raman detection of purine or pyrimidine bases separated from the target nucleic acid. The claims are drawn to a method of detecting a nucleotide or nucleoside comprising: (a) separating a purine or pyrimidine base from the sugar moiety of the nucleotide or nucleoside, (b) depositing the separated base on a surface enhanced Raman spectroscopy (SERS) substrate, (c) synthesizing a double-stranded molecule comprising the separated base on the SERS substrate, and (d) detecting the separated base using SERS. The invention is in a class of invention that the CAFC has characterized as "the unpredictable arts such as chemistry and biology." Mycogen Plant Sci., Inc. v. Monsanto Co., 243 F.3d 1316, 1330 (Fed. Cir. 2001).

Quantity of Experimentation and Unpredictability in the Art

Case law has established that "(t)o be enabling, the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without 'undue experimentation.'" *In re Wright* 990 F.2d 1557, 1561. In *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970) it was determined that "(t)he scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art." The amount of guidance needed to enable the invention is related to the amount of knowledge in the art as well as the predictability in the art.

In the instant case, neither the prior art nor the specification, teaches that a double-stranded molecule is synthesized from a separated nitrogenous base deposited on a SERS substrate. The prior art teaches that a double-stranded nucleic acid is comprised of two

complementary nucleic acid strands. Each single strand of the double-stranded molecule consists of a chain of nucleotides covalently linked through covalent phosphodiester bonds between the sugar moiety of one nucleotide and the phosphate of the next nucleotide. The two strands are held together by hydrogen bonds between complementary purine and pyrimidine bases (see Berg, pages 118-123, for a general description of nucleic acid structure). The prior art does not teach how a double-stranded molecule could be synthesized from a single-stranded molecule and a separated base that lacks the sugar and phosphate groups. Based on the guidance in the prior art, the separated base could participate in non-covalent hydrogen bonding with the single-stranded nucleic acid molecule, but covalent addition of additional nucleotides to form a double-stranded molecule could not occur due to the absence of the sugar and phosphate groups.

This lack of guidance in the art (as well as the specification, discussed below) causes the experimentation required by the skilled artisan to be considered undue. That is, the experimentation to determine this property (i.e., that a double-stranded nucleic acid can be synthesized from a separated nitrogenous base and a single-stranded nucleic acid) requires a trial and error analysis, with little to no starting point, absent any teaching in the specification or the art. In fact, the art actually teaches away from such a synthesis by teaching that a double-stranded molecule requires covalent sugar-phosphate interactions to link the nucleotides comprising each single-stranded molecule and non-covalent hydrogen bonds between the two single strands (see above). Therefore, synthesis of a double-stranded molecule from a single-stranded nucleic acid and a separated base would be considered unpredictable, and is therefore considered to require undue experimentation. In essence, the experimentation that one skilled in the art would be required to perform is in fact the proposed novelty of the invention.

Guidance in the Specification & Working Examples

Although the prior art appears to teach away from synthesizing a double-stranded nucleic acid from a separated base and a single-stranded nucleic acid, MPEP notes "(I)t is the specification, not the knowledge of one skilled in the art that must supply the novel aspects of the invention in order to constitute adequate enablement". (*Genetech Inc. v Novo Nordisk* 42 USPQ2d 1001). Here, the specification also only provides guidance related to conventional synthesis of double-stranded nucleic acid molecules. In other words, the double-stranded molecules taught by the specification contain two chains of nucleotides, where the individual chains comprise nucleotides covalently linked to one another via phosphodiester bonds, and the two single-stranded nucleic acid chains form the double-stranded molecule via non-covalent hydrogen bonding interactions between the nitrogenous bases (see, for example, paragraphs 66, 106, and 125). The working examples also do not provide the necessary guidance. Example 1 teaches SERS detection of a separated nitrogenous base, but does not teach synthesis of a double-stranded molecule comprising the separated base. Examples 3 and 4 teach nucleic acid sequencing methods comprising the following steps: (a) immobilization of a primer, (b) hybridization of the target to the primer, (c) extension of the primer using a polymerase and nucleotides, and (d) detecting unincorporated nucleotides. In these working examples, the double-stranded molecule is synthesized using nucleotides consisting of a nitrogenous base, a sugar moiety, and a phosphate group rather than only a nitrogenous base.

Level of skill in the art

The level of skill is deemed to be high.

Conclusion

Given the unpredictability of the art where synthesis of a double-stranded nucleic acid is not known to be possible using only a separated nitrogenous base and a single-stranded nucleic acid, the large quantity of research required to demonstrate such a synthesis is possible, the lack of guidance provided in the specification, and the absence of any useful working examples balanced only against the high skill level in the art, it is the position of the examiner that it would require undue experimentation for one of skill in the art to perform the method of the claim as written.

Claim Rejections - 35 USC § 112 – Written Description (New Matter)

3. Claims 1-23, 45, and 46 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection.

Claim 1 recites the new limitation "synthesizing a double-stranded molecule comprising the separated purine or pyrimidine base and a single-strand target molecule on the SERS substrate". Figures 1-9 and paragraphs 88-90 of the specification were cited in the response (see page 9) as providing support for this limitation. A careful review of the entire specification including the cited portions did not reveal support for the above limitation. Specifically, the specification fails to teach that purine or pyrimidine bases are separated from the sugar moiety and subsequently incorporated into a growing double-stranded molecule comprising the

separated base and a single-stranded target molecule. The specification teaches synthesis of a double-stranded nucleic acid molecule from an immobilized single-stranded target using a primer, polymerase, and added dNTPs (nucleotides), but does not teach incorporation of nucleotide bases separated from the sugar moiety (see, for example, paragraphs 34, 66, 78, 81, and 90; see also Examples 3 & 4). Furthermore, it is not clear how the separated base can be incorporated to form a double-stranded nucleic acid (except non-covalently via hydrogen bonding between the base and its complement on the single-stranded target) since the sugar moiety has been removed. Therefore, claims 1-15 and 45 contain new matter since the disclosure fails to provide support for the new limitation.

Claims 16-23 and 46 are rejected for incorporating new matter, because claim 16 recites the new limitation "detecting Raman scattering from the double-strand molecule using surface enhanced coherent anti-Stokes Raman spectroscopy (SECARS), to detect a sequence of the single strand target molecule" in step (c). However, the disclosure only teaches detection of unincorporated nucleotides as a method of indirectly detecting the incorporated base(s) and not detection of the newly synthesized double-stranded nucleic acid (see for example, paragraph 66 and Examples 3 and 4). The specification teaches that the observed Raman signal is generated from the substrate-deposited unincorporated nucleotides rather than the newly synthesized double-stranded molecule, and therefore, fails to provide support for the new limitation making a new matter rejection appropriate.

The amendment to claim 16 also presents an additional new matter issue regarding dependent claims 18-23. These claims recite that the single stand target molecule is (or consists essentially of) a nucleotide, a nucleoside or a base. Although the specification provides support

for depositing these molecules on a SERS substrate for subsequent detection, support is not present for synthesis of a double-stranded molecule using only a nucleotide, only a nucleoside, or only a base as the single-stranded template (see Figure 1, paragraphs 90-91, and Examples 3 & 4, where a double-stranded molecule is synthesized from an immobilized nucleic acid single-stranded template (multiple covalently linked nucleotides)). Therefore, claims 18-23 also contain new matter.

Claim Rejections - 35 USC § 103

4. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

5. Claims 16, 17, and 46 are rejected under 35 U.S.C. 103(a) as being unpatentable over Williams et al. (US 6,255,083 B1) in view of Vo-Dinh (US 5,306,403) and further in view of Liang et al. (Chemical Physics Letters (1994) 227: 115-120).

The instant claims are drawn to a method of nucleic acid sequencing comprising depositing a single-stranded target molecule on a surface enhanced Raman spectroscopy (SERS) substrate, synthesizing a complementary double-stranded molecule, and detecting Raman scattering from the newly synthesized double-stranded molecule using surface enhanced coherent anti-Stokes Raman spectroscopy (SECARS).

Williams teaches a fluorescence-based single molecule sequencing method utilizing a microflow chamber to continuously flow doubly labeled NTPs past an immobilized target undergoing primer extension.

Regarding claim 16, the method of Williams (see column 2, lines 15-58) comprises:

(a) obtaining a single-stranded target molecule (column 2, lines 15-24; see column 4, lines 23-32 for further description of the target)

(b) depositing the target on a solid support (column 2, lines 15-24)

(c) synthesizing a double-stranded molecule comprising a complementary purine base and pyrimidine base and the single stranded target on the solid support (col. 2, lines 15-32)

(d) detecting the fluorescence signal from the double-stranded molecule to determine the sequence of the target nucleic acid molecule (column 2, lines 32-40).

Regarding claim 17, Williams teaches that the single-stranded target is isolated from a biological sample (column 4, lines 23-32).

Regarding claim 46, Williams teaches that the detection step monitors a differential concentration of a purine or pyrimidine base before and after synthesizing the.double-stranded molecule (see Example 4, column 17-18). In Example 4, Williams teaches single-molecule sequencing of the CFTR gene, where a known concentration of doubly-labeled fluorescent dNTPs are sequentially incorporated and the released dye was detected (see column 18, lines 10-14). Since the number of molecules sequenced was known, as was the concentration of the dye added, the method of Williams inherently includes a differential concentration measurement, since unincorporated dNTPs are fluorescently silent.

Williams teaches fluorescence detection rather than SECARS detection.

Vo-Dinh teaches a method of DNA sequencing using SERS (see abstract) and particularly points out that the results obtained from fluorescence-based sequencing methods may be misleading since they rely upon signals from labels that display broad, structureless, and overlapping spectra (column 2, line 67 – column 3, line 9).

Liang reports the experimental observation of SECARS. Liang teaches that surface enhancement (SECARS) produced a Raman signal significantly enhanced relative to CARS with improved signal-to-noise (see abstract).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to substitute Raman detection for fluorescence detection in the primer extension-based sequencing method taught by Williams. Vo-Dinh expressly taught that SERS detection was preferable to fluorescence detection in DNA sequencing applications, because fluorescence-based methods inherently suffer from inaccuracies due to the fact that many commonly used fluorescent dyes display broad, structureless, and overlapping spectra (see above). Then, since

the CARS method, which results from excitation with dual lasers, was known to produce a greater signal relative to spontaneous Raman scattering (the source of the SERS signal), the person of ordinary skill would have been motivated to combine surface enhancement with the CARS technique as suggested by Liang in order to improve the sensitivity of the SERS detection method taught by Vo-Dinh. Therefore, an ordinary practitioner of the sequencing method taught by Williams, interested in improving the accuracy and sensitivity of the method, would have been motivated to substitute surface-enhanced Raman detection for fluorescence detection, as suggested by Vo-Dinh, and specifically SECARS, as suggested by Liang, thus resulting in the instantly claimed method.

6. Claims 16 and 17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Xue et al. (US 6,972,174 B2) in view of Vo-Dinh (US 5,306,403) and further in view of Liang et al. (Chemical Physics Letters (1994) 227: 115-120).

The instant claims are drawn to a method of nucleic acid sequencing comprising depositing a single-stranded target molecule on a surface enhanced Raman spectroscopy (SERS) substrate, synthesizing a complementary double-stranded molecule, and detecting Raman scattering from the newly synthesized double-stranded molecule using surface enhanced coherent anti-Stokes Raman spectroscopy (SECARS).

Xue teaches a single nucleotide polymorphism (SNP) genotyping method comprising primer extension on array of immobilized primers using different mixtures of nucleotides and identification of the SNP based on the length of the extension product.

Regarding claim 16, the method of Xue comprises (column 3, lines 6-36):

(a) obtaining and immobilizing a primer on a solid support (col. 3, lines 10-14)

(b) synthesizing a double-stranded molecule comprising a complementary purine or pyrimidine base and the single-stranded target on the substrate (see column 3, lines 15-23, where primer extension occurs on the immobilized target via incorporation of at least one distinguishably labeled NTP)

(c) detecting the signal arising from the incorporated nucleotide to determine the sequence of the target nucleic acid (column 3, lines 15-35).

Regarding claim 17, Xue teaches that the single-stranded nucleic acid target is derived froma biological sample (column 9, lines 51-55, where genomic DNA is purified from a sample).

Xue teaches fluorescence detection rather than SERS detection.

Vo-Dinh teaches a method of DNA sequencing using SERS (see abstract). Vo-Dinh expressly teaches that the results obtained from fluorescence-based sequencing methods may be misleading since they rely upon signals from labels that display broad, structureless and overlapping spectra (column 2, line 67 – column 3, line 9).

Liang reports the experimental observation of SECARS. Liang teaches that surface enhancement (SECARS) produced a Raman signal significantly enhanced relative to CARS with improved signal-to-noise (see abstract).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to substitute Raman detection for fluorescence detection in the primer extension-based sequencing method taught by Xue. Vo-Dinh expressly taught that SERS detection was preferable to fluorescence detection in DNA sequencing applications, because fluorescence-based methods inherently suffer from inaccuracies due to the fact that many commonly used

fluorescent dyes display broad, structureless, and overlapping spectra (see above). Then, since the CARS method, which results from excitation with dual lasers, was known to produce a greater signal relative to spontaneous Raman scattering (the source of the SERS signal), the person of ordinary skill would have been motivated to combine surface enhancement with the CARS technique as suggested by Liang in order to improve the sensitivity of the SERS detection method taught by Vo-Dinh. Therefore, an ordinary practitioner of the sequencing method taught by Xue, interested in improving the accuracy and sensitivity of the method, would have been motivated to substitute surface-enhanced Raman detection for fluorescence detection, as suggested by Vo-Dinh, and specifically SECARS, as suggested by Liang, thus resulting in the instantly claimed method.

7. Claims 24-28, 30-37, 39-44, 47, and 48 are rejected under 35 U.S.C. 103(a) as being unpatentable over Melamede (US 4,863,849; newly cited) in view of Kneipp et al. (US 2002/0150938 A1) and further in view of Vo-Dinh (US 5,306,403).

The instant claims are drawn to a primer extension-based method of nucleic acid sequencing. In the method, a single-stranded target or primer is immobilized on a surface followed by primer extension using a known concentration of a first nucleotide. Upon completion of the extension reaction, the unincorporated nucleotides in the post-reaction mixture are deposited on a SERS substrate, and the resulting SERS signal is used to determine whether or not the nucleotide was incorporated into the target sequence. This procedure is then repeated with different nucleotides to determine the sequence of the target nucleic acid.

Melamede teaches a primer extension-based method of nucleic acid sequencing (see Figures 1-3 and column 11, line 20 – column 14, line 27 for a general description).

Regarding claims 24 and 34, the method of Melamede comprises:

(a) contacting a detectable number of single-stranded template nucleic acid with a mixture comprising a primer, a polymerase, and a known initial concentration of a first nucleotide, where the primer or single-stranded target is immobilized on a surface of the reaction chamber (column 6, lines 39-44 teach adding the reaction mixture to the single-stranded template; column 12, line 58 – column 13, line 3 teach that the initial concentration of the first dNTP is known; column 12, lines 26-30 teach immobilization of the primed template; see also Figures 2 & 3)

(b) annealing the primer to the single-stranded template nucleic acid (column 6, lines 39-44; see also column 12, lines 26-33)

(c) synthesizing a double-stranded molecule comprising the first nucleotide and the single-stranded template (column 6, lines 39-49; see also column 12, line 58 – column 13, line 3)

(d) depositing the post-reaction mixture on a substrate (column 12, lines 39-45), or alternately, flowing the post-reaction mixture past a detector (column 11, line 66 – column 12, line 16; see also column 12, line 58 – column 13, line 3)

(e) detecting a concentration of the first nucleotide using absorption or fluorescence spectroscopy, radioactive labeling, or electrochemical detection (see column 6, lines 48-66 and column 14, lines 15-29).

Regarding claims 25 and 26, Melamede teaches that the concentration of the first nucleotide is greater than the concentration of the single-stranded template, and specifically

teaches using a first dNTP concentration four times greater than the single-stranded template concentration (column 12, line 58 – column 13, line 3).

Regarding claim 27, Melamede teaches adding additional first nucleotide to the reaction mixture after detecting the concentration of the first nucleotide (column 13, lines 19-25; see also column 15, lines 55-64).

Regarding claims 30 and 35, Melamede teaches repeating the process of claim 24 using a different nucleotide (column 15, line 55 – column 16, line 2, where dGTP is the first nucleotide and dCTP is the 2nd nucleotide; see also Figure 2).

Regarding claims 32, 33, 43, and 44, Melamede teaches inclusion of an internal control and comparing the intensity observed from the internal control to that observed from the first nucleotide in order to determine which nucleotide has been incorporated into the template (see column 10, lines 7-10, where inclusion of multiple, different dNTPs is taught; the dNTPs not expected to be incorporated (col. 10, lines 9-10 teach that the template sequence is known) are internal controls).

Regarding claim 36, Melamede teaches washing the substrate (column 6, lines 48-54).

Regarding claim 37, Melamede teaches that the reaction time is about 1 second to about 10 minutes (see column 16, lines 23-38, where the SEQ program is cited; the program has a default reaction time of 1.5 (see line 420 in column 17/18)). Either 1.5 seconds or 1.5 minutes for the reaction time is within the claimed range.

Regarding claims 39 and 40, Melamede teaches that a decrease in the signal intensity of the first nucleotide in the post-reaction mixture compared to the expected value (i.e. the pre-reaction value) identifies the extension product (column 12, line 58 – column 13, line 3). In

determining the expected signal, Melamede inherently performed a pre-reaction analysis of the first nucleotide.

Regarding claim 41, Melamede teaches that the method is performed multiple times for the target position using dATP and dGTP as the added nucleotides (see for example, column 10, lines 1-5).

Regarding claim 42, Melamede teaches that either strand of a double-stranded nucleic acid molecule may be sequenced (see, for example, column 8, lines 46-48).

Regarding claims 47 and 48, Melamede teaches that the detection is by monitoring a differential concentration of a purine or pyrimidine base before and after the synthesizing of the double-stranded molecule (column 12, line 58 – column 13, line 3).

Melamede teaches detection using fluorescence or absorbance spectroscopy, radioactive labeling and counting, or electrochemical detection (column 10, lines 18-24), but does not teach detection using SERS.

Kneipp teaches a SERS-based method of nucleic acid sequencing (see abstract and paragraph 20 for a general description. Briefly, the method of Kneipp comprises the following steps: (a) labeling a nucleic acid sequence with a Raman label (paragraph 42), (b) cleaving a base from the nucleotide (paragraphs 63-64), (c) depositing the separated base on a SERS-active substrate (paragraphs 63 and 48), and (d) detecting the separated base using SERS, thereby sequencing the nucleic acid (paragraphs 63 and 57).

Regarding claim 28, Kneipp teaches sequencing of DNA or RNA by cleaving a base from a nucleotide and detecting the base using SERS (paragraphs 63-64).

Regarding claim 31, Kneipp teaches labeling with a Raman label prior to SERS detection (paragraph 42).

Vo-Dinh teaches a method of DNA sequencing using SERS (see abstract). In the method of Vo-Dinh, SERS labeled nucleic acid fragments are cleaved from a target nucleic acid sequence and detected using SERS (column 6, lines 45-52, for example). Vo-Dinh expressly teaches that fluorescence-based sequencing methods may be misleading since they rely upon signals from labels that display broad, structureless and overlapping spectra (column 2, line 67 – column 3, line 9).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to substitute Raman detection, specifically SERS detection, for the fluorescence detection taught by Melamede. Kneipp taught a SERS-based nucleic acid sequencing method that was highly sensitive, with single molecule detection capabilities (paragraphs 62-64). Vo-Dinh expressly taught that SERS detection was preferable to fluorescence detection in DNA sequencing applications, because fluorescence-based methods inherently suffered from inaccuracies due to the fact that many commonly used fluorescent dyes display broad, structureless, and overlapping spectra (see above). An ordinary practitioner would have been motivated by the teachings of Kneipp and Vo-Dinh to substitute SERS detection for the fluorescence detection taught by Melamede in order to improve the accuracy and sensitivity of the method. Regarding the relative concentrations of the dNTP (claims 25 and 26), Melamede expressly taught using an excess (4 equivalents) of the first dNTP in the reaction (column 12, line 58 – column 13, line 3). Although the claimed limitations of: (a) equal amounts of target and dNTP or (b) a dNTP concentration twice as large as the target concentration were not

explicitly taught in the above references (Melamede, Kneipp, Vo-Dinh), an ordinary practitioner would have recognized that the target and NTP concentrations were not critical provided that the functional limitations (ability to reliably detect separated and/or newly incorporated nucleotides) were satisfied and would have optimized target and nucleotide concentrations as necessary in order to achieve the best results. As noted In re Aller, 105 USPQ 233 at 235:

> More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.

Routine optimization is not inventive and no evidence has been provided to suggest that the use of the particular target and NTP concentrations was anything other than routine or that the results were unexpected as compared to the closest prior art. Therefore, the combined teachings of Melamede, Kneipp, and Vo-Dinh result in the method of the instant claims 24-28, 30-37, 39-44, 47, and 48.

8. Claim 29 is rejected under 35 U.S.C. 103(a) as being unpatentable over Melamede (US 4,863,849; newly cited) in view of Kneipp et al. (US 2002/0150938 A1) and further in view of Vo-Dinh (US 5,306,403) and further in view of Liang et al. (Chemical Physics Letters (1994) 227: 115-120).

The combined teachings of Melamede, Kneipp, and Vo-Dinh result in the method of claim 24, as discussed above.

None of the above references teach detection using SECARS.

Liang reports the experimental observation of SECARS. Liang teaches that surface enhancement (SECARS) produced a Raman signal significantly enhanced relative to CARS with improved signal-to-noise (see abstract)

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to utilize SECARS detection in the method resulting from the combined teachings of Melamede, Kneipp, and Vo-Dinh. Since Liang taught that the CARS method, which results from excitation with dual lasers, produced a greater signal relative to spontaneous Raman scattering (the source of the SERS signal), the person of ordinary skill would have been motivated to combine surface enhancement with the CARS technique, as suggested by Liang, in order to improve the sensitivity of the SERS detection method taught by Vo-Dinh and Kneipp. Since the only required modification to the SERS-based detection would have been incorporation of CARS laser sources, the person of ordinary skill would have expected a reasonable level of success in substituting SECARS for SERS. Therefore, the ordinary practitioner of the SERS detection method resulting from the combined teachings of Melamede, Kneipp, and Vo-Dinh, interested in obtaining a more sensitive detection method, would have been motivated to substitute SECARS detection as suggested by Liang, thus resulting in the instantly claimed methods.

9. Claim 38 is rejected under 35 U.S.C. 103(a) as being unpatentable over Melamede (US 4,863,849; newly cited) in view of Kneipp et al. (US 2002/0150938 A1) and further in view of Vo-Dinh (US 5,306,403) and further in view of Quake (US 6,002,471).

The combined teachings of Melamede, Kneipp, and Vo-Dinh result in the method of claim 34, as discussed above.

None of the above references teaches a reaction chamber with at least one dimension less than 100 nm.

Quake teaches a high resolution scanning Raman microscope capable of nanometer level sequencing of DNA based on Raman signals (see abstract and column 2, lines 63-65).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to utilize a nanoscale detection device as taught by Quake in the combined method of Melamede, Kneipp, and Vo-Dinh in order to decrease sample requirements and improve the resolution of the method. Quake taught that SERS resolution was increased and sample requirements decreased relative to the prior art of Kneipp, for example (see column 1, lines 38-59 and column 2, lines 6-21). Since the device taught by Quake was specifically designed for Raman-based DNA sequencing applications, the ordinary user would have been motivated to apply the teachings of Quake, thereby resulting in a reaction chamber having a reduced size (less than 100 nm in at least one dimension, for example), thus resulting in the instantly claimed method.

Double Patenting

10. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection

is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

11. Claims 16-23 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 16-23 of copending Application No. 11/020,776 in view of either Williams (US 6,255,083 B1) or Xue et al. (US 6.972,174 B2).

Claims 16-23 of the '776 application recite a SECARS-based nucleic acid sequencing method highly similar to the method of the instant claims 16-23. The instant claims differ from those of the '776 application in two ways: (1) the instant claims specify that the target is single-stranded and (2) claim 16 of the instant application includes an additional step of synthesizing a double-stranded molecule from the single stranded target not found in claim 16 of the '776 application.

As discussed in greater detail above, Williams and Xue separately teach a primer extension-based nucleic acid sequencing method, wherein a target is hybridized to an immobilized primer followed by primer extension and fluorescence detection of the double-stranded target. An ordinary practitioner of the method recited in claims 16-23 of the '776

application would have been motivated by the teachings of Williams or Xue perform the sequencing method using a single-stranded target followed by detection of the newly synthesized double-stranded molecule. Williams and Xue taught that the method, in which a signal was only generated upon correct incorporation of a complementary base, was highly sensitive and specific. An ordinary practitioner would have been motivated to synthesize a double-stranded molecule from a single-stranded target prior to detection by Raman spectroscopy in order to increase the specificity of target detection. Therefore, the method of the instant claims 16-23 is obvious in view of claims 16-23 of the '776 application in view of either Williams or Xue.

This is a provisional obviousness-type double patenting rejection.

12. Claims 24-44 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 24-44 of copending Application No. 11/020,776 in view of Melamede (US 4,863,849).

Claims 24-44 of the '776 application recite a SERS-based nucleic acid sequencing method highly similar to the method of the instant claims 24-44. The instant claims differ from those of the '776 application in two ways: (1) the instant claims specify that the target is single-stranded and (2) claims 24 and 34 of the instant application include an additional step of synthesizing a double-stranded molecule from the single stranded target not found in the corresponding claims 24 and 34 in the '776 application.

As discussed in greater detail above, Melamede teaches a primer extension-based sequencing method. Melamede taught that the primer extension mixture was analyzed post-reaction, and a decrease in signal intensity of a particular nucleotide indicated that the nucleotide

had been incorporated into the primer (see above). The method of Melamede utilized an immobilized single-stranded target and included a step of synthesizing a double-stranded target from the single-stranded template and a complementary base (see above). Melamede taught that the method was sensitive, specific, and readily automated (see column 7, lines 17-28). An ordinary practitioner of the method taught in claims 24-44 of the '776 application would have been motivated to synthesize a double-stranded molecule from a single-stranded target as taught by Melamede in order to obtain the advantages taught by Melamede (increased specificity, accuracy, automation). Therefore, the method of the instant claims 24-44 is obvious in view of claims 24-44 of the '776 application in view of Melamede.

This is a provisional obviousness-type double patenting rejection.

Response to Arguments

13. 35 U.S.C. 112 rejections

Applicant's arguments, see page 9, filed July 26, 2006, with respect to the rejection of claims 13-15 as indefinite under 35 U.S.C. 112, 2nd paragraph have been fully considered and are persuasive. Applicant's amendment to the claims has corrected the antecedent basis problem, and accordingly, this rejection has been withdrawn.

35 U.S.C. 102 rejections

Applicant's arguments, see page 9, filed July 26, 2006, with respect to the rejection of claims 1-7 under 35 U.S.C. 102(b) as being anticipated by Kneipp, have been fully considered

and are persuasive. Kneipp does not teach all of the elements of the amended claim 1, and accordingly, this rejection has been withdrawn.

35 U.S.C. 103 rejections

Applicant's arguments with respect to claims 8-44 have been considered but are moot in view of the new ground(s) of rejection presented above.

Double Patenting Rejections

The previously made provisional statutory double patenting rejection of claims 1-44 as claiming the same invention as copending application 11/020,776 has been withdrawn, because the amended claims no longer recite the same invention as the '776 application. However, claims 16-23 of the '776 application in combination with either the Williams or Xue reference render the instant claims 16-23 obvious (see the new obviousness-type double patenting rejection above). Also, claims 24-44 of the '776 application in combination with Melamede render the instant claims 24-44 obvious (see the new obviousness-type double patenting rejection above).

Regarding the previously made obvious-type double patenting rejections with copending applications 10/660,902, 10/108,128, 11/270,211, and 11/255,386, the amendment to the instant claim 1 has overcome the rejection. Therefore, these rejections have been withdrawn.

Conclusion

No claims are currently allowable.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Kunkel et al. (US 2003/0077584 A1) teaches a nucleic acid sequencing method

comprising sequential detection of unincorporated nucleotides following a primer extension reaction.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Angela Bertagna whose telephone number is (571) 272-8291. The examiner can normally be reached on M-F 7:30-5 pm EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Angela Bertagna
Examiner, Art Unit 1637
October 5, 2006

amb

KENNETH R. HORLICK, PH.D
PRIMARY EXAMINER

10/5/06